Identification of bile salt hydrolase inhibitors, the promising alternative to antibiotic growth promoters

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Antibiotic growth promoters (AGPs)

- AGPs: a group of antibiotics used as feed additives (low dose) in food animal industry for more than five decades.
- AGPs can improve daily weight gain and feed efficiency of various food animals.

Food safety and public health concerns for AGP usage

- Emergence and prevalence of drug-resistant foodborne pathogens are correlated with the use of AGPs.
- AGP ban
  - 1998 in Denmark
  - 2006 European Union
- To date, there is a worldwide trend of limiting AGP use in food animals.
- Ending the use of AGPs creates challenges for the animal feed industries.

Mode of action of AGPs

- The precise mechanisms are still not clear.
- It is widely accepted that AGP usage affects gut microbiota and results in an optimal and balanced microbiota for enhanced growth performance.
- Examination of the effect of AGP on intestinal microbiota is important for developing novel alternatives to AGPs.

AGP usage and chicken intestinal microbiota

- Experimental diet:
  - Non-medicated (control)
  - Medicated-
    - Day 1-32→ salinomycin & bacitracin
    - Day 33-42→ virginiamycin
- Body weights determined on days 7, 14, 21, 32, 42.
  - At day 14, 32 and 42, one bird from each pen whose body weight was nearest the mean for the pen was removed for intestinal sample collection.

Effect of AGP on gut microbiota

Culture-independent approaches

- Phylochip

- 16S rDNA libraries analysis

Luminal fecal samples collected at day 42 from ileum of 8 birds (4 control, 4 medicated) were used for rDNA library construction (768 clones).

Bacterial identity		Number of Clones	
Order	**Genus**	**Control**	**Medicated**
Lactobacillales	*Lactobacillus*	45	0
Lactobacillales	*Leuconostoc*	1	0
Lactobacillales	*Streptococcus*	3	0
Erysipelotrichales	*Turicibacter*	199	70
Clostridiales	*Peptostreptococcaceae*	120	197
	Total	369	267

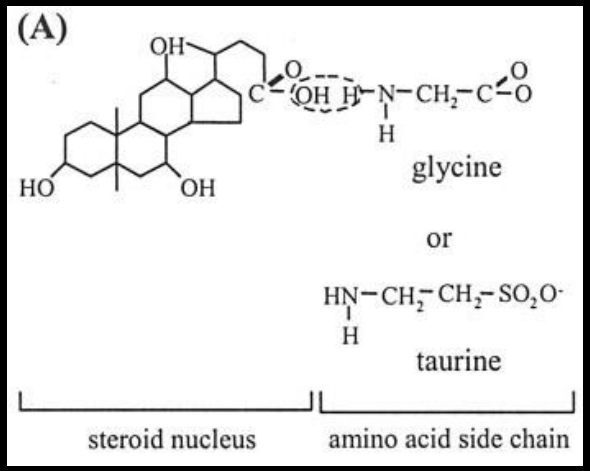
AGPs reduced the population of *Lactobacillus* in the chicken intestine

- *L. salivarius*: the dominant lactic acid bacterium present in the intestine
- *Lactobacillus* species are the major commensals that produce bile salt hydrolase (BSH) in the intestine.

1) Dumonceaux et al. 2006. *Appl Environ Microbiol* 72:2815-2823.
2) Engberg et al. 2000. *Poult Sci* 79:1311-1319.
3) Guban et al. 2006. *Poult Sci* 85:2186-2194
4) Knarreborg et al. 2002. *Appl Environ Microbiol* 68:5918-5924.
5) Zhou et al. 2007. *Poult Sci* 86:2541-2549

Bile Salt Hydrolase (BSH)

- Bile salts: synthesized in the liver and conjugated with either glycine or taurine prior to secretion
- The conjugated bile salts are needed to maintain efficient lipid digestion and absorption
- Function of BSH



Begley et al. 2006. Appl Envion Microbiol. 72:1729

Conjugated bile salts

BSH

Unconjugated bile salts + glycine/ taurine

The growth-promoting effect of AGPs was highly correlated with the decreased BSH activity in the intestine

- Feighner & Dashkevicz, 1987. *Appl Environ Microbiol* **53:**331-336.
- Knarreborg et al., 2004: *J Nutr* **134:**1487-1492.
- Guban et al., 2006: *Poult Sci* **85:**2186-2194.

Hypothesis

Inhibition of BSH activity using specific inhibitors is a promising approach to promote feed efficiency and weight gain in food animals

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0099-2240/87/020331-06$02.00/0

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Subtherapeutic Levels of Antibiotics in Poultry Feeds and Their Effects on Weight Gain, Feed Efficiency, and Bacterial Cholyltaurine Hydrolase Activity

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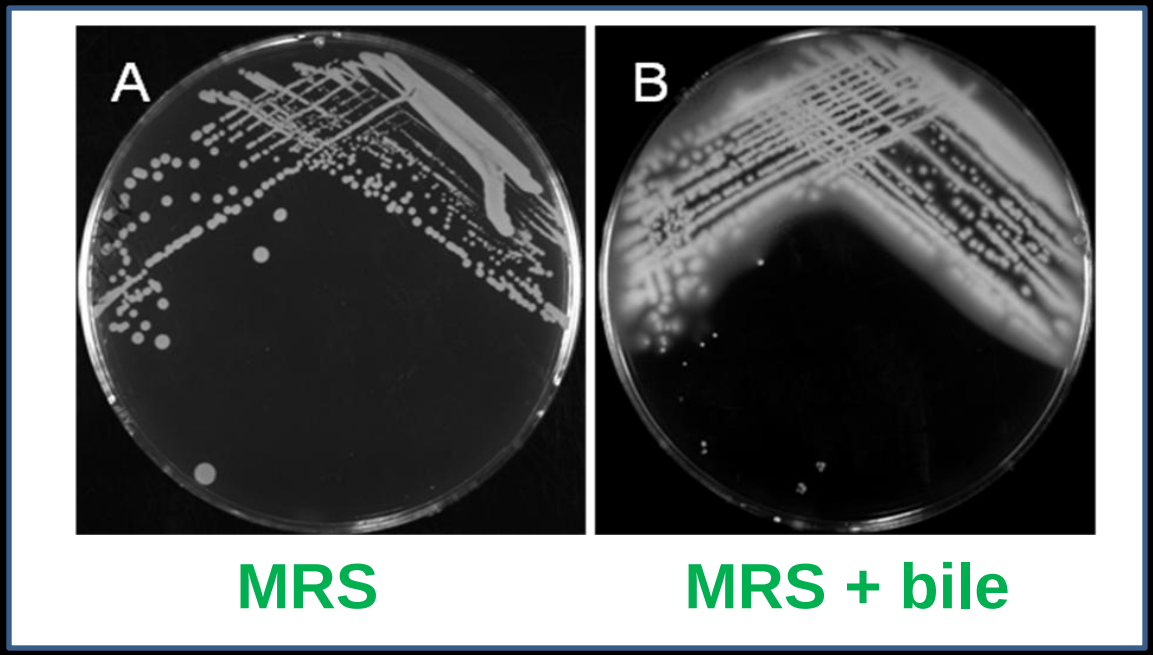
A radiochemical method was developed to estimate cholyltaurine hydrolase potentials and rates of cholyltaurine hydrolysis in chicken intestinal homogenates. This method was used to monitor the effects of antibiotic feed additives on cholyltaurine hydrolase activity. Avoparcin, bacitracin methylenedisalisylic acid, efrotomycin, lincomycin, penicillin G procaine, and virginiamycin improved rate of weight gain and feed conversion of chicks and decreased cholyltaurine hydrolase activity in ileal homogenates relative to those of nonmedicated control birds. The results provided the first evidence that feeding selected antibiotics at subtherapeutic levels can affect bile acid-transforming enzymes in small-intestinal homogenates. The inverse relationship between growth performance and cholyltaurine hydrolase activity raises the possibility that specific inhibitors of this enzyme may promote weight gain and feed conversion in livestock and thereby reduce or eliminate the need for antibiotic feed additives.

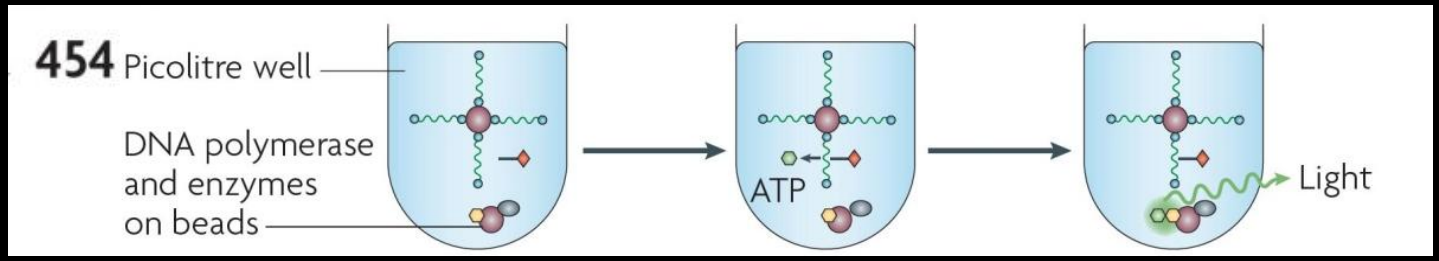
Lactobacillus salivarius NRRL B-30514

- A chicken isolate that produces bacteriocin
- Display potent BSH activity to hydrolyze conjugated bile salts



Stern et al. 2006. Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacter jejuni* in the chicken gastrointestinal system. *Antimicrob Agents Chemother* **50:**3111-3116.

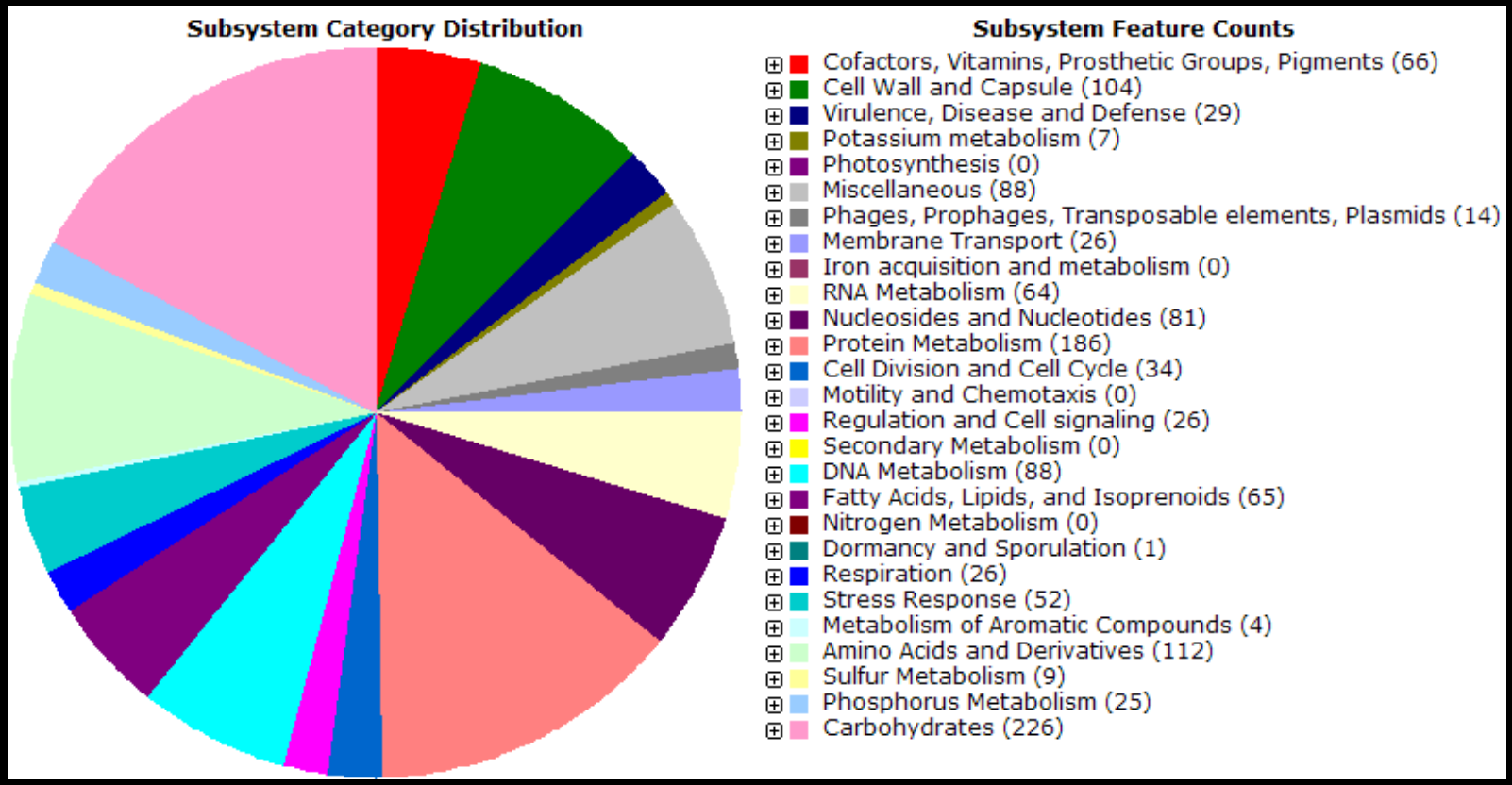
Whole Genome Sequencing of *L. salivarius* NRRL B-30514 (454 FLX Titanium)



Total number of reads	**238,829**
Total number of bases	**96,071,065**
Average read length	**357**
No. of total bases in all contigs	**1,913,653**
No. of total bases in large contigs	**1,892,975**
No. of all contigs	**108**
No. of large contigs	**47**
Size (bp) of large contigs	**502 to 170,991**
Average coverage depth fold	**50**

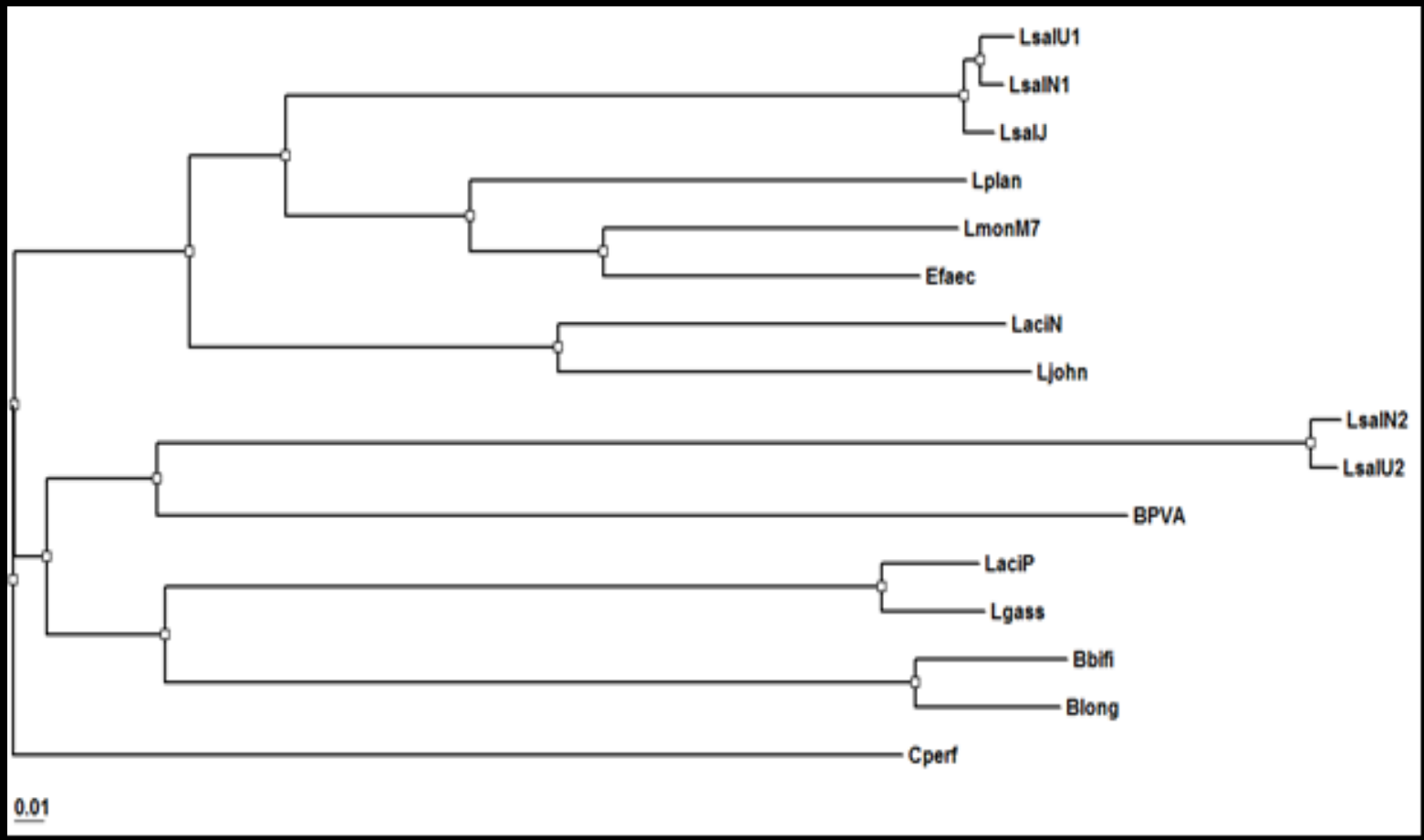
Automatic annotation by the RAST server (http://rast.nmpdr.org/)

of ORFs: 1878

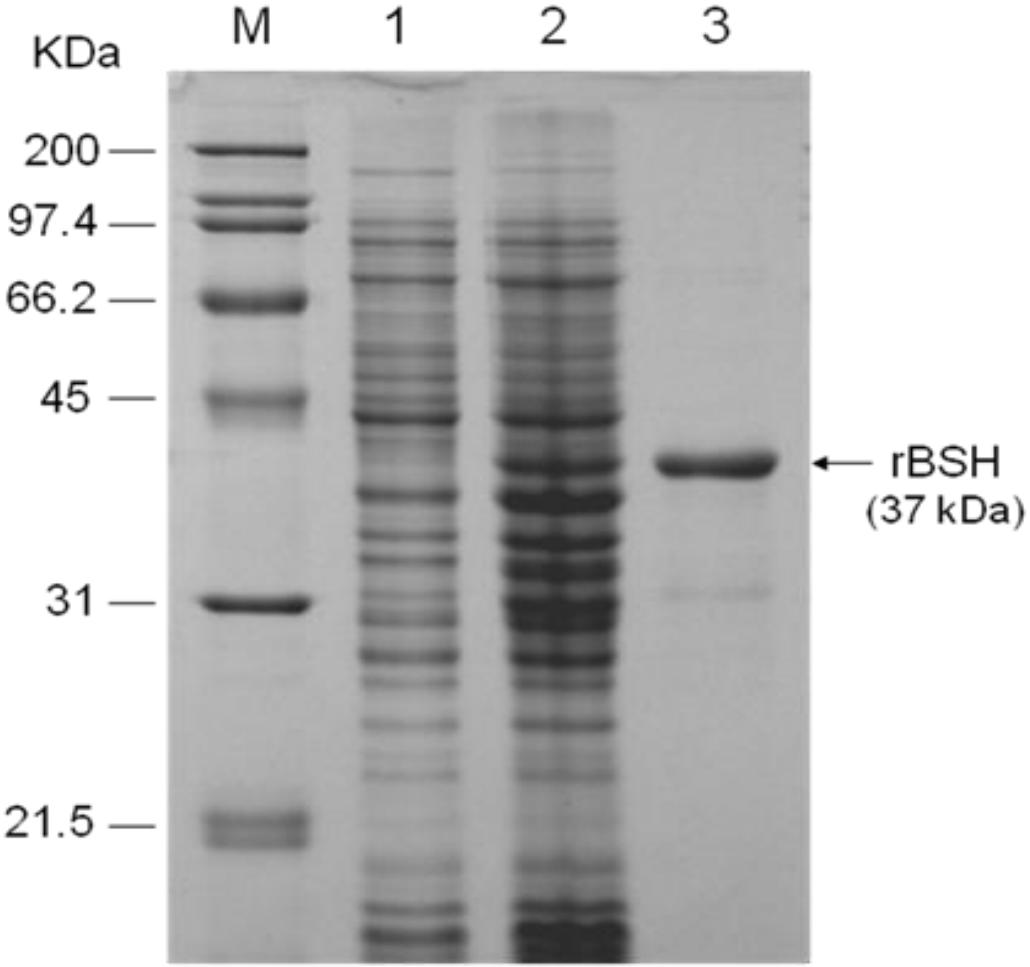


Identification of two BSH genes

- BSH1: contig 107
- BSH2: contig 7



Expression and Purification of rBSH



Lane 1: Cell lysate w/o IPTG induction.

Lane 2: Cell lysate w IPTG induction

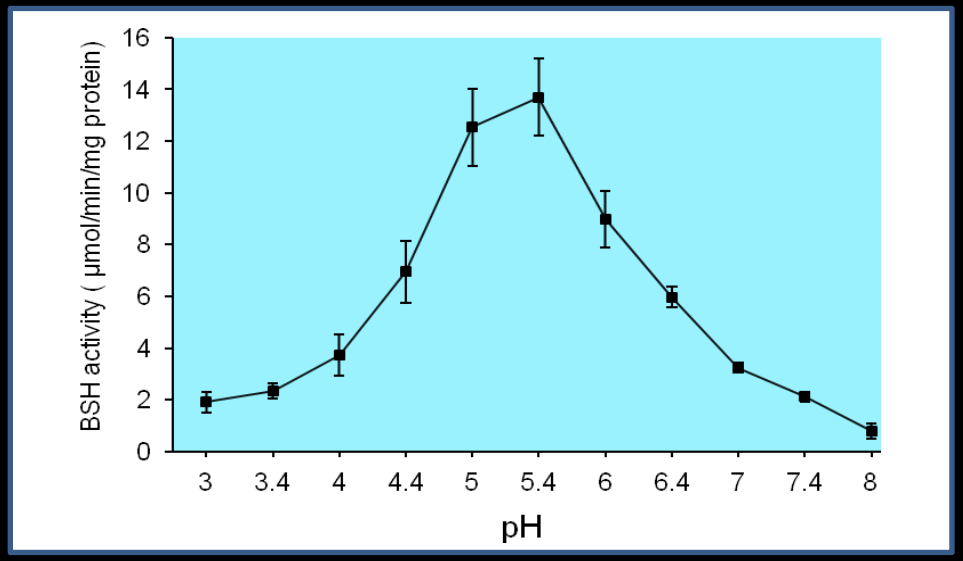
Lane 3: Purified His-tagged rBSH

Activity and kinetics of the rBSH for different bile salts

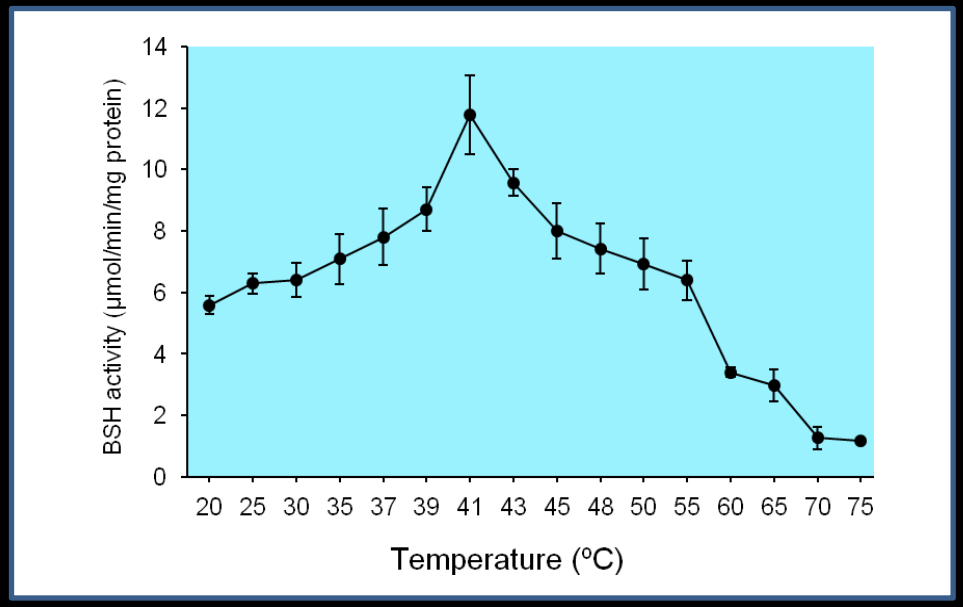
Substrate	BSH activity μmol/min/mg	Relative activity (%)	K_m(mM)	k_{cat} (min^{-1})	k_{cat}/K_m ($min^{-1}mM^{-1}$)
GCA	7.7±0.48	41.9	1.71	532	311
GDCA	4.0±0.54	22.3	1.15	382	332
GCDCA	17.7±1.18	100	2.48	938	378
TCA	5.6±0.33	31.4	3.21	585	182
TDCA	8.5±2.26	47.9	3.19	806	252
TCDCA	8.0±2.37	45.1	2.53	510	201

The BSH displayed efficient hydrolysis activity for both glycoconjugated and tauroconjugated bile salts

Effect of pH and Tm on BSH Activity



BSH maximum activity occurred at pH 5.5



The BSH has the highest activity at around 41°C

Inhibitory effect of various feed additives on the BSH activity

Compound	% Inhibition		Compound	% Inhibition
$CuCl_2$	98.1		$CuSO_4$	91.7
$ZnCl_2$	68.3		$ZnSO_4$	89.5
$MnCl_2$	68.1		$MnSO_4$	83.1
$FeCl_3$	73.0		$FeSO_4$	96.1
KCl	25.9		$NaSeO_3$	93.1
NaCl	27.7		$NaSO_4$	27.7
$MgCl_2$	25.7		$MgSO_4$	31.3
$NaIO_3$	88.8		KIO_3	92.9
$CoCl2_2$	95.9		$NaHCO_3$	20.6
$CaCl_2$	22.4		Vitamin C	21.8

Copper/zinc have been used at high concentrations to aid in feed efficiency and growth promotion

- Poultry
  - Arias and Koutsos. 2006. *Poult Sci* 85:999-1007.
  - Ewing et al. 1998. *Poult Sci* 77:445-448.
  - Miles et al. 1998. *Poult Sci* 77:416-425.
- Swine
  - Armstrong et al. 2004. *J Anim Sci* 82:1234-1240
  - Hill et al. 2000. *J Anim Sci* 78:1010-1016.
  - Jacela et al. 2010. *Journal of swine health and production.* 18:87-91.
  - Smith et al. 1997. *J Anim Sci* 75:1861-1866.

Discovery of potent, safe, and cost-effective BSH inhibitors

- Screen more compounds including emerging feed additives (e.g. dietary plant bioactives)
- High-throughput screening (HTS)
  - We have developed a rapid, convenient, and effective HTS system.

Conclusions

- Identified and characterized a BSH with broad substrate specificity from a chicken *L. salivarius* strain.
- Established a solid platform for us to discover novel BSH inhibitors, the promising feed additives to replace AGPs for enhancing the productivity and sustainability of food animals.

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Questions?